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Assessment of Taste Threshold by Forced Choice Method of Ten *Madhuraskandha* Drugs

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Abstract

Background: *Rasa* (taste) determination is one of the directly perceivable parameter for the identification of drugs, while *Veerya* (potency) and *Vipaka* (bio-transformation) are inferable. These principles help to predict the action of the drug in Ayurveda. Taste threshold method evolved by C Dwarakanath helps to determine the dominant *Rasa* in the drug. **Aim:** To find out the dominance of the *Madhurarasa* (Sweet taste) in the 10 drugs belonging to *Madhuraskandha* (group of sweet taste predominant drugs). **Materials & Methods:** The study was conducted by single blind, forced choice method in 60 (6×10) female healthy volunteers. All the samples were administered in hot and cold dosage forms. The volunteers were asked to note down the start point and threshold point of taste perception by using Likert gradation scale in specially designed proforma. The obtained data were analyzed by applying appropriate statistical methods. **Results:** The statistically significant difference at the threshold point taste perception was found in case of sample *Vidari, Eranda, Gokshura*, and *Guduchi* with both dosage forms when compared to reference standard drug. **Conclusion:** The intensity of *Rasa* of *Madhuraskandha* drug can be categorized into mild, moderate and severe (*Taratamattva*) by comparing with reference standard drug. In the ten selected sample, *Vidarikanda* and *Eranda* may be classified into Mild; *Gokshura* as Moderate; *Kantakari, Guduchi, Shalaparni* and *Shatavari* with severe intensity of the *Rasa*.

Keywords: *Madhuraskandha drugs, Forced choice method, Likert scale (Taste threshold)*

Introduction

Rasa is an important quality manifested at the level of tongue and is the only principle or quality which is directly perceivable. *Panchabhuatic* (five basic elements) combination and primary physical qualities or *Gunas* (qualities) of different *Rasas* are mentioned in classics. Though it appears that *Gunas* are intimately related to *Rasas* it is a fact that both are separate principles co-existent in the *Dravyas*[1]. *Acharya* Priyavrat Sharma had discussed about the concept of *Rasa* basing on the physico-chemical constitution of substances. E.g. *Madhurarasa* contains carbohydrates, amino acids and all the *Amlarasa Dravyas* show acidic reaction etc [2].

The *Acharyas* of *Brihattrayi* had adopted different criteria for classification of drugs viz. basing on the *Karya-Karana* (cause and effect),*Chetana-Achetana* (activity), *Rasa* etc. However *Acharya* Charaka had implemented this criteria in the context of *Asthapana bastidravya* (Corrective enema). He had explicitly stated that the drugs included in the group (*Rasaskandha*) are grouped mainly based on *Rasa* and *Rasa* oriented *Vipaka* and *Prabhava* (factors responsible for specific action). E.g. *Madhuraskandha* contains drugs having *Madhurarasa* or *Madhuravipaka* or *Madhuraprabhava*.

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Prof. C Dwarakanath attempted to evolve a method known as "Taste threshold" for quantitative determination of intensity of *Rasa* in a given substance. The test is meant for evaluation of the intensity or degree of the taste which finally depends on the tongue only. The taste perception and sensibility are complex bio-physical and psychological events and translation of *Rasa* cannot be exactly evaluated without the help of tongue. In the present study, ten drugs were selected from the *Madhuraskandha* classification of Charaka. They were studied to find out the dominance of the *Madhurarasa* in these drugs by forced choice method in healthy volunteers.

Materials and Methods:

Collection of drugs:

All the drug samples were procured according to part used and they were cleaned, washed and shade dried (Table 1). All the drugs were powdered and stored in the air tight container. The drugs were authenticated in the Laboratory of Pharmacognosy, I.P.G.T&R.A, Jamnagar.

Preparation of drug:

Each drug was administered in two dosage forms: cold infusion and hot infusion.

Cold infusion: Five grams of sample was soaked in 100 ml of distilled water, stirred for 30 min. It was filtered with filter paper and used for the experiment. The similar procedure was followed for each sample.

Hot infusion: Five grams of sample was soaked in 100 ml of hot distilled water. It was stirred for 30 min and filtered. The filtrate was used for the experiment.

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Selection of volunteers:

The M.D and Ph.D. scholars, having knowledge of *Rasa* perception, of female sex either in pre-ovulatory and post-ovulatory period, were selected from the Institute for Post Graduate teaching & research in Ayurved, Jamnagar. The volunteers having health problems like stomatitis, acute rhinitis, fever etc. or having addiction of smoking, chewing tobacco or betel nut in which taste perception gets impaired, male volunteers and menstruating females were excluded. The study was carried out in 6 female volunteers for each drug (n=6×10=60).

Study design:

The study followed single blind method. Drugs were coded as per mentioned in Table 1

Table 1 Details of Ten selected Drugs of *MadhuraSkandha*

Sample	Botanical source	Part used
S1	*Atibala* *Abutilon indicum* Linn. Sweet.	Root
S2	*Vidari* *Pueraria tuberosa* DC	Tuber
S3	*Kantakari* *Solanum xanthocarpum* S. &W	Whole plant
S4	*Eranda* *Ricinus communis* Linn	Root
S5	*Gokshura* *Tribulus terestris* Linn	Fruit
S6	*Guduchi* *Tinospora cordifolia* (Willd.) Miers ex Hook. f. &Thoms	Stem
S7	*Shalaparni* *Desmodium gangeticum* DC.	Root
S8	*Jivanti* *Leptadenia reticulata* W. & A.	Leaves
S9	*Shatavari* *Asparagus racemosus* Willd.	Tuberous root
S10	*Punarnava* *Boerhavia diffusa* Linn	Root

Assessment of taste threshold:

The taste threshold of the drug was determined by following method of dilution (S.C. Dhyani, 1977) with slight modification[3]. It is ascending and rating scale method. Before commencing the main experiment, pilot study had been carried out in 5 volunteers to decide the range of dilution for each drug.

Dilution preparation:

The cold and hot infusion of each drug sample were serially diluted. Dilution was made by adding 1ml of filtrate of cold infusion in 25 ml of water which is numbered as 1:25 dilution, likewise further dilutions were made with the difference of 10-20 ml, till the taste disappeared, which is considered as threshold point of that drug. The same method was followed for the hot infusion dosage form of the drug. In this study, table sugar (sucrose with sweetening index 1) was taken as reference standard drug.

Threshold detection:

The volunteers were asked to refrain from tea, coffee or any food items before half an hour of the experiment. They were instructed to rinse the mouth with water. Each volunteer was subjected for tasting approx. 1 ml of each dilution starting from lower concentration to higher concentration (ascending method). They were advised to note down their expressions based on Likert scale[4]. The gradation of scale is as described in Table 2. By applying this scale starting point and end point or threshold point of the taste perception has been decided. The observation

Table 2 Rating-scale of taste descriptions

Response	Likert (numerical values used in analysis but never shown to subjects)
1	Same as water
2	Doubtful if pure water
3	A very faint taste, can't say
4	A very faint taste (mention the name of taste)
5	A faint taste (mention the name of taste)
6	A weak taste (mention the name of taste)
7	Clear taste (mention the name of taste)

obtained were noted down in designed proforma basing on the memory about different tastes of the individuals.

Statistical analysis:

The obtained data were analyzed by calculating the mean of the dilutions. Comparison between two dosage forms of each drug was evaluated by applying unpaired 't' test and comparison between standard drug and test drug has been done by applying one way ANOVA followed by Dunnett's test by using Sigma stat 3.5 version.

Observation & Result:

In this study, six female volunteers were selected for each drug sample [5]. Each drug sample was evaluated for two dosage form, i.e. Cold and Hot infusion. The volunteers were asked to note down the start and threshold point of taste perception.

The data depicted in the table 3 shows the starting point and end point of the taste perception. The taste perceived by dilution method was not only *Madhura* but also *Tikta, Kashayarasa* (Bitter and astringent taste) observed along with *Madhurarasa*. The combination of *Madhura, Tikta* were found in S2, S3, S5, S6 and S9, while *Madhura, Kashayarasa* were noticed in S4, and S8. The samples S1, and hot infusion of S10 were having *Tikta* and *Kashayarasa. Madhura, Tikta* and *Kashayarasas* were found in both the dosage forms of S7. The drugs having *Madhurarasa* were compared with reference standard drug.

The table 4 shows the comparison between standard drug and test sample at the end point or threshold point. There is statistically significant difference at the threshold point taste perception in case of sample S2, S4, S5, and S6 with both dosage forms when compared with reference standard drug.

Table 3 showing observations made by volunteers for taste threshold (n=6)

Sample	Dosage form	Start point (Clear taste) (ml)	Threshold point (Same as water) (ml)	Taste
S1	Cold	76.66±9.18	186.67±4.94	*Tikta, Kashaya*
	Hot	74.16±14.40	191.66±3.07	*Tikta, Kashaya*
S2	Cold	13.33± 4.01	98.33±4.77	*Madhura, Tikta*
	Hot	22.00±7.93	103.33±5.57	*Madhura, Tikta*
S3	Cold	116.66±11.15	243.33±8.02	*Madhura, Tikta*
	Hot	100±10	225±8.06	*Madhura, Tikta*
S4	Cold	20.83±5.83	100±7.30	*Madhura, Kashaya*
	Hot	36.66±5.57	120±5.16	*Madhura, Kashaya*
S5	Cold	68.33±8.72	205±7.18	*Madhura, Tikta*
	Hot	68.33±8.72	180±5.77	*Tikta, Kashaya*
S6	Cold	100.83±17.72	213.33±6.66	*Madhura, Tikta*
	Hot	79.16±14.28	176.66±8.43	*Madhura, Tikta*
S7	Cold	74.16±15.29	265.00±21.25	*Madhura, Tikta, Kashaya*
	Hot	75.83±16.35	201.66±8.33	*Madhura, Tikta, Kashaya*
S8	Cold	97.50±20.64	306.67±30.40	*Madhura, Kashaya*
	Hot	65.00±26.86	271.66±30.81	*Madhura, Kashaya*
S9	Cold	106.66±20.11	246.67±11.15	*Madhura, Tikta*
	Hot	85.00±12.84	213.33±12.56	*Madhura, Tikta*
S10	Cold	43.33±12.01	166.66±11.15	*Tikta, Madhura*
	Hot	35.00±10.24	153.33±12.29	*Tikta, Kashaya*
Ref	Cold	51.66±19.39	266.67±22.90	*Madhura*
	Hot	50.83±19.03	270.00±22.94	*Madhura*

Data: Mean±SEM

The data described in the table 5 shows the comparison between standard drug and test sample at the starting point. The sample S3 has shown statistically significant difference in starting point of taste perception when compared to reference standard drug.

The comparison between both the dosage forms of individual drugs has been mentioned in the table 6. The taste perception with cold and hot infusion of test

Table 4 showing comparison between standard drug and test drugs at threshold point

Sample	Dosage	Threshold point	SD	F
S2	Cold	98.333±4.773***	11.69	51.782
	Hot	103.333±5.578***	13.663	49.801
S3	Cold	243.33±8.028	19.664	0.925
	Hot	225.00±8.062	19.748	3.423
S4	Cold	100.00±7.303***	17.889	48.077
	Hot	120.00±5.164***	12.649	40.663
S5	Cold	205.00±7.188*	17.607	6.601
	Hot	180±5.774***	56.214	14.464
S6	Cold	213.33±6.667*	16.33	5.00
	Hot	176.667±8.433**	20.656	14.572
S7	Cold	265.00±21.252	52.058	0.00285
	Hot	201.667±8.333*	20.412	7.833
S8	Cold	306.67±30.405	74.476	1.104
	Hot	271.667±30.813	75.476	0.0018
S9	Cold	246.67±11.155	27.325	0.616
	Hot	213.33±12.561	30.768	4.692
Ref	Cold	266.67±22.901	56.095	-
	Hot	270.00±22.949	56.214	-

Data: Mean ±SEM, *p<0.05, **p<0.01, ***p<0.001 by comparing the test drugs with standard drug by applying one way ANOVA followed by Dunnett's test

sample has shown no statistically significant difference.

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Discussion:

One of the earliest characteristics of human sensory function to be measured was the absolute threshold. The absolute or detection threshold was seen as an energy level below which no sensation would be produced by a stimulus and above which a sensation would reach consciousness. American Society for Testing and Materials (ASTM) provides the definition for chemical senses as 'A concentration range exists below which the odor or taste of a substance will not be detectable under any practical circumstances, and above which individual with normal sense of smell or taste would readily detect the presence of a substance'[6]. There are various physiological factors which affects the taste perception, e.g. Age [7] (older men are less sensitive than young men and women for acetic acid, sucrose, citric acid, sodium and potassium chloride), Sex [5] (The increase in sensitivity of the women during pre-ovulation may have been related to

Table 5 showing comparison between reference and test drugs at starting point

Sample	Dosage	Starting point	SD	F
S2	Cold	13.33± 4.01	9.832	3.74
	Hot	22.00±7.93	19.42	1.88
S3	Cold	116.66±11.15*	27.32	8.44
	Hot	100.00±10.00*	24.49	5.22
S4	Cold	20.83±5.83	14.28	2.31
	Hot	36.66±5.57	13.66	0.51
S5	Cold	68.33±8.72	21.37	0.61
	Hot	68.33±8.72	21.37	0.69
S6	Cold	100.83±17.72	43.40	3.50
	Hot	79.16±14.28	34.98	1.41
S7	Cold	74.16±15.29	37.47	0.83
	Hot	75.83±16.35	40.05	0.99
S8	Cold	97.50±20.64	50.57	2.61
	Hot	65.00±26.86	65.80	0.18
S9	Cold	106.66±20.11	49.26	3.87
	Hot	85.00±12.84	31.46	2.21
Ref	Cold	51.66±19.39	47.50	-
	Hot	50.83±19.03	46.62	-

Data: Mean ±SEM, *p<0.05, when compared with reference standard drug by one way ANOVA test

Table 6 showing comparison of taste perception between two dosage forms of individual drug

Sample	Likert scale observations		SD		t	p
	Cold	Hot	Cold	Hot		
S1	3.08±0.44	3.06±0.35	1.66	1.33	0.041	0.967
S2	2.94±0.37	2.81±0.37	1.30	1.28	0.236	0.815
S3	3.06±0.30	3.34±0.31	1.18	1.17	-0.635	0.531
S4	2.79±0.40	2.59±0.46	1.39	1.61	0.321	0.751
S5	3.22±0.36	2.56±0.38	1.41	1.48	1.240	0.225
S6	2.79±0.35	2.66±0.36	1.42	1.41	0.245	0.808
S7	3.43±0.39	2.83±0.38	1.57	1.54	1.095	0.282
S8	2.55±0.26	2.34±0.24	1.14	1.08	0.583	0.563
S9	2.84±0.23	3.31±0.36	0.90	1.41	-1.077	0.291
S10	2.50±0.44	2.56±0.41	1.48	1.37	-0.099	0.922
Ref	2.52±0.36	2.61±0.32	1.50	1.34	-0.201	0.842

Data: Mean ±SEM, p value calculated when both dosage forms of each drug compared separately (Unpaired t test) the high level of estrogen, whereas it is comparatively lower during post ovulatory phase), hunger sensation (Taste sensitivity for sweet and salty taste increases), pregnancy [8] (salty taste sensitivity decreases), addiction like smoking, plasma levels of leptin, serotonin, noradrenaline and also pathological conditions like diabetes [9], adrenal cortical hormone deficiency [10] causes alteration in taste perception.

When the taste of the drug is explicit, then there shall be no doubt about the perception of taste. But two or more *Rasas* manifest in the drug with almost equal quantity or intensity, both the tastes may be perceived and it may be difficult to interpret which is the dominant or *Pradhana Rasa* (dominant taste). In such cases, taste perception with tongue method may give difference of opinion. Therefore, the taste perception by dilution method helps to solve this problem.

The ten selected drugs from the *Madhuraskandha* can be categorized into mild, moderate, and severe basing upon the intensity of the taste in the drugs by comparing with standard drug. After comparing their threshold points, the sample *Vidarikanda* and *Eranda* may be classified into Mild; Sample *Gokshura* as Moderate; Sample *Kantakari, Guduchi, Shalaparni* and *Shatavari* categorized into higher intensity of *Rasa*.

These drugs are found to be *Madhura* and *Tiktarasa* dominant. They are found with higher concentration of *Rasa* because it is observed that bitter taste follows the sweet taste and intensity of *Tiktarasa* is higher than any other rasa. (The threshold for stimulation of bitter taste of Quinine is 0.000008M)[11].

The drugs tasted with cold and hot infusion didn't show any statistically significant difference. Both the dosage forms were found to be similar in threshold point perception. Hence these drugs can be used along with cold and hot water.

The sample S8 contains Leptadenia reticulata with part used as leaves. Leaves consist of chlorophyll (coloring agent) and also have leafy aromatic odor. However, there have been many other studies showing enhancement of specific taste qualities, notably sweetness, in the presence of odors. An important tendency, especially among untrained customers is to misattribute some volatile olfactory sensations to 'taste', particularly retro nasally perceived odors. Retro-nasal smell is poorly localized and often perceived as a taste from the oral cavity. A common finding is that when drugs are deeply colored, they will obtain higher rating for flavor intensity[12]. Hence, sample S8 was found with higher threshold point as compared to other samples.

Conclusion:

Taste threshold assessment by using dilution method helps to confirm the intensity of *Rasa* in the drug. The use of Likert scale makes a volunteer to captures the intensity of their feelings for a given item in particular statement. The drugs selected from the *Madhuraskandha* have taste other than *Madhurarasa*. *Atibala* and *Punarnava* have shown the dominance of *Tikta, Kashayarasa*, while other samples have *Madhurarasa* along with either *Tikta* or *Kashayarasa* or both. The higher degree of *Rasa* was found in *Kantakari*, *Shatavari*, *Guduchi* and *Shalaparni* with dominance of *Madhura* and *Tiktarasa*.

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